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Potential Hazards of Fumigant Residues

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A *spectrum of fumigants (primarily ethylene dibromide, 1,2-dibromo-3-chloropropane,* ethylene oxide, *sym*dibromotetetrachloroethane, 1,3-dichloropropene, dichlorovos, carbon tetrachloride, methyl bromide) as well as their degradation products in foodstuffs and soil have been examined mainly in regard to the potential mutagenicity of their residues.

Fumigants are gaseous pesticides that are widely employed in considerable amounts for the control primarily of insects, mites, nematodes, wireworms, rodents and to a lesser extent bacteria, yeasts and molds in stored foodstuffs. Table 1 lists 27 of the most commonly used fumigants, including a number of alkylating, carcinogenic, and mutagenic agents, potential carcinogens and mutagens, and hepatotoxins. In addition, a variety of fumigant mixtures are employed (e.g., carbon tetrachloride–ethylene dichloride–ethylene dibromide; methyl bromide–ethylene dibromide–carbon tetrachloride; carbon tetrachloride–acrylonitrile). Knowledge of the nature and amount of the fumigant residues *per se* and/or their degradation products (primarily in foodstuffs and the atmosphere) is of obvious vital concern. The primary objective of this overview is to highlight a small number of illustrative problem areas dealing with a spectrum of mutagenic and potentially mutagenic fumigant residues.

The factors influencing the distribution and persistence of a specific fumigant and/or its degradation product(s) are complex and can be illustrated in the 13 factors depicted in Table 2 as applied to a consideration in grain (*1-3*). Equally complex factors are manifest concerning the fate of fumigants and their degradation products in soil (*4-6*).

Residues of ethylene dibromide (EDB) (as well as inorganic bromide) have been reported in a wide range of fruits, vegetables (*7*), flour, bran, and middlings as well as fumigated packaging materials with residues of EDB remaining in the latter for up to 6 weeks after fumigation (*8*). Ethylene dibromide has been shown to be mutagenic in *Neurospora crassa* (*9*), *Tradescantia* (*10*), *Salmonella typhimurium* (TA 1530) (*11*) and preferentially inhibits the growth of DNA

Table 1. Commonly used fumigants.

Fumigant	Structure
Acrylonitrile	$CH_2=CHCN$
Benzene	C_6H_6
Carbon disulfide	CS_2
Carbon tetrachloride	CCl_4
Chloroform	$CHCl_3$
Chloropicrin	CCl_3NO_2
sym-Dibromotetrachloroethane	$Cl_2BrC\text{-}CCl_2Br$
1,2-Dibromo-3-chloropropane	$BrCH_2CHBrCH_2Cl$
1,3-Dichloropropene	$ClCH_2\text{-}CH=CHCl$
Dichlorvos	$(CH_3O)_2P(O)OCH=CCl_2$
Ethyl formate	$HCOOCH_2CH_3$
Ethylene dibromide	$BrCH_2CH_2Br$
Ethylene dichloride	$ClCH_2CH_2Cl$
Ethylene chlorobromide	$BrCH_2CH_2Cl$
Ethylene oxide	CH_2CH_2 (O)
Hydrogen cyanide	HCN
Methyl bromide	CH_3Br
Methylene chloride	CH_2Cl_2
Naphthalene	$C_{10}H_8$
p-Dichlorobenzene	$C_6H_4Cl_2$
Perchloroethylene (tetrachloroethylene)	$Cl_2C=CCl_2$
Phosphine	PH_3
Propylene oxide	$H_3C\text{-}CH\text{-}CH_2$ (O)
Sulfur dioxide	SO_2
Sulfuryl fluoride	SO_2F_2
1,1,1-Trichloroethane	CH_3CCl_3
Trichloroethylene	$ClCH=CCl_2$

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Table 2. Factors influencing the distribution and persistence relationships of fumigant cases of volatile liquids applied to grain.

	Factor
1.	Nature of the fumigant
2.	Applied dosage
3.	Nature of the substrate
4.	Dockage content(foreign matter)
5.	Moisture content
6.	Absolute humidity
7.	Vapor pressure
8.	Temperature
9.	Diffusion and atmospheric pressure
10.	Interstitial atmospheric composition
11.	Chemical and physical sorption affinities of the fumigants
12.	Air movement patterns affected by temperature gradients
13.	Chromatographic properties of the grain substrates

polymerase-deficient (pol A-) *E. coli* (*12*). Ethylene bromohydrin is a potential metabolite of both ethylene oxide and ethylene dibromide fumigation. For example, Heuser and Scudamore (*13, 14*) reported the formation of ethylene bromohydrin in flour and wheat treatment with ethylene oxide, in which the required bromine ions derived either from naturally occurring inorganic bromide or in larger amounts from bromide produced in prior fumigation with methyl bromide. Harvested crops, notably tobacco, may also contain inorganic bromide derived from soil treatment with methyl bromide or ethylene dibromide.

Ethylene bromohydrin has been found to be mutagenic in *Klebesiella pneumoniae* (*15*), *S. typhimurium* (TA 1530) (*16*), and inhibits the growth of DNA-polymerase deficient *E. coli* (*16*).

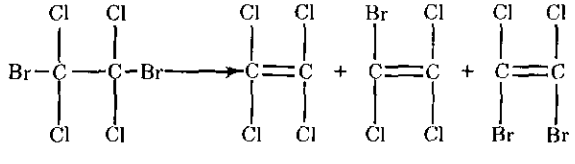
It has been suggested by Olson et al. (*17*) that chronic exposure to the fumigants ethylene bromide or 1,2-dibromo-3-chloropropane (DBCP) could be a health hazard. This would be most relevant to agricultural and food storage workers who disperse these volatile agents through soil or food with the hazard to any such workers probably being manifest primarily via inhalation rather than oral exposure. The induction of stomach cancer in rats and mice following chronic oral intubation of ethylene dibromide and 1,2-dibromo-3-chloropropane was reported by Olson et al. (*17*). It was suggested that in either EDP or DBCP, the bromine atoms are activated so that the compounds probably act as alkylating agents. Current concepts implicate alkylation of DNA, RNA, or other macromolecular cell constituents as one mechanism of carcinogenesis (*18*) as well as mutagenesis (*11*).

Ethylene oxide is widely used as a fumigant to sterilize foodstuffs, textiles, medical instruments, as well as in the tobacco industry to shorten the aging process and to reduce the nicotine content of tobacco leaves (*19,20*). The toxicity and mutagenic potential of ethylene oxide are well documented (*20*). For example, ethylene oxide is mutagenic in *Drosophila* (*21,22*), *Neurospora* (*23,24*), and barley (*25*), and includes chromosome aberrations in maize (*26*), barley (*27*), and *Vicia faba* (*28*).

It has been found that under certain conditions proportions for effective fumigation, ethylene oxide reacts with moisture and chloride ions to form ethylene chlorohydrin (2-chloroethanol), a relatively nonvolatile (bp 129°C) toxic substance which has been detected in a variety of foodstuffs (*19,29,30*) in ppm amounts. Ethylene chlorohydrin has been reported to be mutagenic in *Klebsiella pneumoniae* (*31*) (TA 1530 and TA 1535) (*32*), and preferentially inhibit the growth of DNA polymerase deficient *E. coli* (*32*).

Symmetric dibromotetrachloroethane (DBTCE) is a broad spectrum fungicide-fungistat that is very effective in the vapor phase for the prevention of sporulation and reduction of decay of citrus caused by the blue-green molds (*33*), the control of *Botrytis cinerea* decay of table grapes in storage and in shipping containers (*34*), and for control of *Fusarium caeruleum* and *Corticum solani* decay on stored potatoes (*35*).

DBTCE is stable in formulations when mixed with binders or extenders such as starch, clay, or air-borne silica. Most reported reactions involve free radical reactions under the influence of strong illumination (it decomposes to greater extent with increasing temperature to bromine and tetrachloroethylene); also under illumination, DBTCE can react with olefins to produce alkylic bromides C_2Cl_4 and HBr (*36*). Extensive decomposition of DBTCE has been reported to take place on citrus peel and in juice (*37*) (orange and lemon). In addition to inorganic bromide, the organic halogen products found were identified as tetrachloroethylene, bromotrichloroethylene, and 1,2-dibromodichloroethylene [eq. (1)].

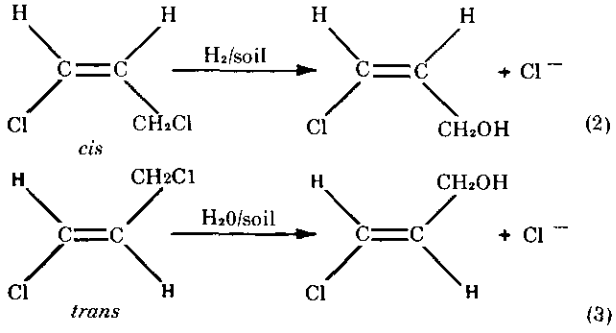


DBTCE (1)

Also identified was bromopentachloroethane (BPCE), an impurity in batches of DBTCE. BPCE was not found to be metabolized on citrus fruit.

The studies of Kolbenzen et al. (*37*) showed that DBTCE used as a vapor phase post-harvest treatment for decay and sporulation reduction on citrus, is rapidly metabolized via dehalogenation (with debromination being the most probable reaction) to bromide ion, tetrachloroethylene and bromotrichloroethylene. At high levels of treatment, traces of dibromodichlorethylene are also found. It can be envisioned that the above products of DBTCE can further react with traces of moisture to produce a host of potentially mutagenic chloro-, bromo-, and/or chlorobromohydrin products.

1, 3-Dichloropropene (Telone) is an effective soil fumigant and nematocide for all croplands. Dorlane is a mixture of dichloropropene and ethylene dibromide. The technical product is a mixture of *cis* and *trans* isomers. *Cis*- and *trans*-1, 2-dichloropropenes are hydrolyzed in wet soil to *cis* and *trans*-3-chloroalkyl alcohols, respectively (*38*), as shown in eqs. (2) and (3). Soil does not inhibit the normal solvolysis (*39*) of *cis*- or *trans*-1, 3-dichloropropene, and the biocidal and possible toxicological properties of the chloroalkyl alcohols must be considered when the dihalide fumigants are employed. It is significant that the chloroalkyl alcohols are rather wide-range biocides (*40*).



Dichlorovos (2, 2-dichlorovinyl dimethyl phosphate) (DDVP) (Vapona) has been employed as an insecticidal fumigant for stored foods and products such as grains and tobacco, for pre- and post-harvest crop treatment and for disinfection of aircraft. It is prepared via the reaction of trimethylphosphate and chloral. It should be noted that these intermediates are mutagenic *per se*, the former inducing point mutations in *Neurospora crassa* (*41*), as well as being highly active in the dominant lethal test (*42*), while the latter has been reported to induce point mutations in *Drosophila* (*43*) and bacteria (*44*) and chromosome aberrations in *Vicia faba* (*45*).

It is of interest to note the usual constituents of Vapona (*46*) when used in resin strips are; DDVP, 95–97%; dipterex (*0,0*-dimethyltrichloro-1-hydroxyethyl phosphate), 1.5–3%; *0,0*-dimethyl-2-chlorovinyl phosphate, 0.4–0.7%; *0,0*-dimethyl phosphonate, trace to 0.1%; *0,0,0*-trimethylphosphate, 0.3–0.8%; chloral (trichloroacetaldehyde), 0.1–0.5%. (In addition, the resin releases small quantities of plasticizers and other materials.)

Recently, the question of absorption of dichlorovos vapors by exposed foodstuffs following disinfection of aircraft has been raised (*47*). Whole meals exposed for 30 min. to 0.25 μg/l. dichlorvos absorbed approximately 0.18 ppm; one-tenth of this concentration was found in beverages similarly exposed, while concentrations in margarine were three times as high. Dale et al. (*47*) calculated on the basis of the above that if one were to consume a 300-g dinner with 5 g of margarine and two beverages after exposure for 30 min. to a concentration of 0.25 μg/l. he would consume approximately 60 μg of dichlorovos. The maximum acceptable daily intake of dichlorvos recommended by FAO/WHO is 0.004 mg/kg or 280 μg for a 70-kg person (*48*).

Howe et al. (*49*) found considerable sorption of DDVP vapor by wheat when it was employed as a fumigant.

Because of the wide exposure of humans to atmospheres containing DDVP as well as the potential of ingestion in food of DDVP and /or its metabolites and degradation products) this agent has been extensively studied, in regard to both its potential alkylating capacity (*50, 51*) as well as its mutagenicity, with conflicting results. Dichlorvos has been shown to alkylate DNA (*51*), with the types of alkylations produced, resembling methyl methane sulfonate (MMS) more closely than other common alkylating agents although DDVP has the additional capacity to dimethyl phosphorylate protein (*52*). On a molar basis, DDVP is about as effective as MMS in alkylating protein but about 15-fold less potent in alkylating DNA (*53*). A dose on the order of 30*mM* of DDVP for 1 hr was computed to produce about 1400 methylations in the *E. coli* WP2 genome.

Bridges et al. (*54*) compared the lethal and mutagenic effects of DDVP with those of MMS in a series of radiation-sensitive strains of *E. coli*. ExrA, RecA, and PolA strains of *E. coli* exhibited increased sensitivity to both agents. Mutagenesis with both DDVP and MMS was demonstrated to occur by misrepair (e.g., ExrA or RecA strains were

not mutated). It was shown that there was much less difference in survival between resistant and sensitive strains with DDVP than with MMS, and DDVP was a much weaker mutagen (*54*).

Wild (*55*) and Mohn (*56*) reported definite mutagenicity and clear relationships between DDVP concentration, exposure time and the mutagenic effect in two other genetic tests in *E. coli*, e.g., induction of streptomycin resistance (5.25*mM* DDVP, 1–10 hr) and of 5-methyltryptophan resistance (0.3–3.2*mM* DDVP, 0.5–5 hr), respectively.

DDVP has been shown to be mutagenic in other bacterial species, e.g., inducing in *Serratia marcescens* with the agar plate test (*57*) and in *Salmonella typhimurium* (*58*). In several bacterial species, including *E. coli, S. typhimurium,* and *Klebsiella pneumoniae,* the mutation rate to streptomycin resistance was increased (*31*).

A dose-dependent increase of mitotic gene conversion by 5–40*mM* DDVP (5 hr) has been demonstrated in the D4 strain of the yeast *S. cerevisiae* (*59, 60*).

Although the quantitative relationship between mutagenesis and alkylation of specific sites in DNA has not yet been established, it should be noted that the doses used in the microbial mutation studies above are of the same order as those used in DNA alkylation studies (*53, 61*).

Michalek and Brochman (*62*), using the adenine-3 region of *Neurospora crassa,* were unable to demonstrate any mutagenicity of DDVP. However, Dean (*57*) has shown that DDVP (technical, >97%) at very high concentrations (25–100 mg/ml in DMSO) is capable of inducing mutation in *Serratia marcescens* under specific *in vitro* conditions. The conditions in the bacterial test systems, in which DDVP is in intimate contact with the cell and hence more readily available to the bacterial DNA are stressed by Dean (*60*) to be vastly different to the situation *in vivo,* where the dichlorvos molecule is confronted by a variety of hydrolytic enzymes (*63*). In this regard, a number of recent studies of Dean and his co-workers are of special importance. For example, Dean and Thorpe (*64*) demonstrated the absence of dominant lethal mutations in male CF_1 mice following single and repeated inhalation exposure to DDVP at concentrations of 30 and 55 μg DDVP/l. for 23 hr daily for 4 weeks. These exposures to DDVP produced no mutagenic effects as expressed by increased preimplantation of early fetal deaths in subsequent test matings, neither was an impairment of male fertility detected following the exposures to DDVP vapor. It was stressed by Dean and Thorpe (*64*) that the DDVP concentration of 5.8 μg/l. DDVP used in their repeated exposure is more than 100 times the average air DDVP concentration of 0.04 μg/l. of air found during the domestic use of dichlorvos-impregnated resin strips.

The failure of high doses of DDVP to induce chromosome damage in mice and Chinese hamsters has also been reported (*65*).

Carbon tetrachloride is widely used alone or in admixture with ethylene dichloride, ethylene dibromide, methyl bromide, carbon disulfide, and acrylonitrile in the disinfestation of stored cereal grains.

Evidence for the existence of measurable quantities of CCl_4 in foodstuffs moving in commerce has been provided by a survey of residues in imported cereals and related products carried out in the Netherlands (*66*). Almost half the samples examined contained detectable amounts of CCl_4 while in 3% of samples, over 5 ppm were recorded. Wit et al. (*67*) reported very small residues of CCl_4 (up to 0.07 ppm) in bread baked from flour treated with a carbon tetrachloride–ethylene dichloride mixture. Scudamore and Heuser (*68*) studied levels of CCl_4 in fumigated cereal grains in England and concluded that complete elimination of trace amounts of CCl_4 from products of treated grain was unlikely even after milling, but the toxicological significance of such residues was uncertain. The 1971 Joint FAO/WHO Meeting (*69*) suggested the limits for carbon tetrachloride should be 50 ppm on raw cereals at the point of entry into a country, 10 ppm in milled cereal products to be subjected to baking or cooking and 0.05 ppm in bread or other cooked cereal products.

Carbon tetrachloride has produced liver tumors in the mouse, hamster, and rat following several routes of administration, including inhalation and oral ingestion (*70, 71*). The prolonged administration of CCl_4 to rats causes centrolobular fatty change and necrosis. Frequent mitotic figures and binucleate cells are evidence of cellular regeneration and hyperplasia (*72–74*).

A number of cases of hepatomas appearing in men several years after CCl_4 poisoning have also been reported (*72*). It has been suggested that the selective toxicity of CCl_4 for the liver of animals depends on its metabolism by the liver (*75, 76*) to a reactive chemical species. For example, it was proposed that a free radical, $CCl_3\cdot$, is formed, and that this could lead to peroxidation of lipid membranes and produce chemical alterations at other sites (*75*). Liver tissue reduces carbon tetrachloride to chloroform, and it was suggested by Butler that homolytic cleavage of the carbon–chlorine bonds

yields free radicals, which could then alkylate the sulfhydryl groups of enzymes. A link between the latter and peroxidative decomposition was established by Rechnagel (*76*). Fowler detected hexachloroethane (CCl_3CCl_3) in tissues of rabbits following CCl_4 intoxication (*77*).

Bartholmess (*78*), in his survey of chemical mutagens in the environment, has listed carbon tetrachloride as a chromosome breaking agent.

Methyl bromide is an alkylating agent widely used *per se* or in combination (e.g., with chloropicrin; ethylene dibromide and carbon tetrachloride) for soil (*79*) and grain fumigation (*80*). Brown et al. (*79*) reported that bromine content of potato tubers grown in soil fumigated with methyl bromide at 487 and 975 kg/ha (1 lb and 2 lb/100 ft^2) averaged 170 and 280 mg/kg of dry weight. Most of the bromine was in the outer layers of the tubers; peeled tubers had less than 100 mg/kg, which remained after boiling. Wheat commonly follows potatoes in the planting rotation and the bromine content of wheat grain grown after potatoes depended on the rate of application of methyl bromide and the time interval between treatment and wheat crop. Wheat grain harvested 3.5, 2.5, and 1.5 yr after fumigation with methyl bromide at 975 kg/ha had mean bromine contents of 4.5, 15, and 44 mg/kg, but the amount in grain from plots having the same treatments varied more than twofold (*79*).

Fumigation of wheat with methyl bromide has been reported by Bridges (*80*) to yield *N*-methylated products such as 1-*N*-methylhistidine, 3-*N*-methylhistidene and 1, 3-*N*, *N*-dimethylhistidine. A joint FAO/WHO working party has recommended (*81*) a maximum acceptable daily intake of 1 mg bromine ion/kg body weight, with a tolerance level of 50 mg/kg in raw cereals or whole meal flour (*81*).

In addition to bromine taken up by crops during growth, fumigation with bromine-containing compounds used to control pests during storage can also increase their bromine content (*82, 83*). As much as 40 mg Br/kg on a dry weight basis was sorbed by wheat grain and larger amounts by milled wheat products. Bromine sorbed during fumigation in storage, when added to that derived from methyl bromide-treated soils during growth, could thus produce bromine contents greater than the recommended tolerance levels, even if grain at harvest contained substantially less (*83*).

There is a paucity of knowledge on the metabolic fate of bromides in man and animals (*81, 82*). Williford et al. (*84*) reported a higher accumulation in tissues, particularly muscle, and the eye in rats fed methyl bromide fumigated diets. Bromine substitution for chloride in muscle, blood, liver, and kidney (*85*), as well as the higher bromine concentrations in animal proteins than in animal fat (*82, 86*) have been reported.

In summary, a broad spectrum of toxic chemicals are employed individually as well as in admixture as fumigants. Many of these fumigants are alkylating agents (e.g., dichlorvos, ethylene dibromide, ethylene oxide, propylene oxide, methyl bromide) and possess mutagenic properties *per se* or via their metabolic and/or degradation products (e.g., dichlorvos, ethylene dibromide, ethylene oxide, ethylene chlorohydrin, ethylene bromohydrin, sulfur dioxide, bisulfite). A number of the commonly employed fumigants are either carcinogenic and/or hepatotoxic (e.g., benzene, carbon tetrachloride, chloroform, ethylene dibromide, 1, 2-dibromo-3-chloropropane, trichloroethylene and tetrachloroethylene).

An *a priori* consideration of the structure of a number of fumigants also suggests their possible mutagenicity, particularly in activated systems, (analogous to vinyl chloride) (*87, 88*). This would be particularly relevant to 1, 3-dichloropropene, perchloroethylene, trichloroethylene, and possibly acrylonitrile, which may all undergo epoxidation, as well as the hydrolysis products of 1,3-dichloropropene (e.g., *cis*- and *trans*-3-chloroalkyl alcohol).

There is an admitted paucity of definitive mammalian toxicological data on many of the employed fumigants as well as their principal metabolic and/or degradation products which may reach man via direct exposure or via ingestion as residues in a broad spectrum of foods.

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